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CARDIAC PERFORMANCE IN RESPONSE TO LOADING PRESSURES AND PERFUSION WITH 5-HYDROXYTRYPTAMINE IN THE ISOLATED HEART OF *BUSYCON CANALICULATUM* (GASTROPODA, PROSOBRANCHIA)

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SUMMARY

In this study the effects of a molluscan neurotransmitter, 5-hydroxytryptamine (5-HT), were examined on the isolated and pumping heart of the gastropod mollusc *Busycon canaliculatum*. Unlike in previous studies, the response was measured in such a way as to equate it with cardiac output. In addition, the effects on the myogram form and the manner of perfusate ejection were also examined.

As would be expected from previous studies, 5-HT affects heart rate, showing a positive chronotropic response at a threshold of around 10^{-9} mol l^{-1}. Stroke volume shows little evidence of being regulated by 5-HT concentration. This observation was unexpected as 5-HT is commonly reported to regulate the 'force' of contraction in a molluscan heart and, at constant perfusion conditions, this might have been expected to find expression as an increase in stroke volume. 5-HT does, however, have a very clear dose-dependent effect on the aortic pressure pulse amplitude and duration. Amplitude increases markedly (250%) over the concentrations used (10^{-10}–10^{-6} mol l^{-1}) with a threshold around 10^{-8} mol l^{-1}. The effect on the duration has the same threshold but the opposite result, with a reduction to approximately 50% of the original value. The same amount of perfusate is therefore being ejected at a higher pressure and flow rate. It is suggested that this might have important implications for a soft-bodied animal with a hydrostatic skeleton.

The electrical activity of the heart was also examined and showed that 5-HT increased the amplitude of both the spike and plateau phase of the action potential. The duration of the latter was reduced. This is discussed with reference to other studies.

INTRODUCTION

Cardiac output is controlled by integrating the autoregulation of the heart and the action of extrinsic factors. In an earlier study (Smith, 1985*a*) on the isolated ventricle of the whelk *Busycon canaliculatum*, alteration of the preload level, as set by the filling pressure, not only regulated output in accordance with Starling's Law but also

Key words: loading pressure, heart, 5-hydroxytryptamine, Mollusca.

modulated the form of the action potential. An increase in the preload caused (1) the prepotential to rise more rapidly and (2) the area under the plateau to increase. For the reasons argued in that study [particularly with reference to the work of Irisawa, Kobayashi & Matsubayashi (1961*a*) and Hill & Irisawa (1967)] myogram records made using a suction electrode were taken to be a faithful representation of the muscle action potential. Several earlier studies, using a number of molluscan species, have also examined the relationship between the configuration of the action potential and the force of contraction (Irisawa *et al.* 1961*a*,*b*; Nomura,1963; Hill & Irisawa, 1967; Hill, 1974*a*,*b*; Hill & Yantorno, 1979). We know, therefore, that the filling pressure is one factor that could modulate the form of the action potential and thus affect the contractile force and cardiac output. Neuroendocrine substances, acting as neurotransmitters or local hormones, may also modulate the form of the action potential and, as a direct result, the performance of the heart.

The early evidence for 5-hydroxytryptamine (5-HT; serotonin) as a molluscan cardioregulatory substance was reviewed by Hill & Welsh (1966) and subsequently by Jones (1983). Liebeswar, Goldman, Koester & Mayeri (1975) identified a serotonergic cardioexcitatory motoneurone in *Aplysia californica*. The characteristic effect of 5-HT on the ventricle of the gastropod *Helix pomatia* (Kiss & S-Rozsa, 1972, 1975) is to prolong the repolarizing phase (the plateau) of the action potential, even at a threshold concentration of 10^{-10} mol l^{-1}. The plateau formation is blocked in the absence of external sodium ($[Na^+]_o$). Thus in *Helix*, as in the bivalve *Modiolus* (Wilkens, 1972*a*,*b*), the sodium current may maintain the level of depolarization during the plateau induced by 5-HT. For the ventricle of the gastropod *Dolabella auricularia*, prolongation of the cardiac action potential by 5-HT is correlated with an increased force of beating (Hill, 1974*a*).

In almost all the pharmacological studies concerning the action of 5-HT on the electrical activity and force of contraction of the molluscan heart, force has been measured in such a way as to make it impossible to equate with cardiac output. This paper describes a series of experiments designed to take advantage of the predictability of the isolated gastropod heart to investigate the action of 5-HT on the two factors which contribute to output: heart rate and stroke volume.

MATERIALS AND METHODS

The species of gastropod used in this study was the whelk *Busycon canaliculatum* (L.). The work was carried out at the Department of Zoology, University of Rhode Island, USA, during June 1984 and April 1985. Animals were acquired either locally or from the Marine Biology Laboratory, Woods Hole, Massachusetts. They ranged in size from 158 to 241 g without shells ($\bar{x} = 211 \pm 33$ g). The mean wet heart weight, measured as described by Smith (1985*a*), was $0{\cdot}42 \pm 0{\cdot}1$ g. Both sexes were used and the experiments were conducted at a temperature of 23 °C.

In order to perfuse a heart at realistic pressure levels (see Smith, 1985*a*) it is necessary to devise a preparation which controls both the input pressure to the heart (the preload) and the output back pressure on the heart (the afterload). In the case of

Busycon, the dissection has already been described in detail by Smith (1985*a*). In summary, an input cannula was installed in the efferent branchial vessel and led to a reservoir whose height could be varied relative to the water level in the experimental chamber. This constitutes the preload level (P1). The output cannula was installed in the anterior aorta and fed to a second reservoir (P2, afterload). In these experiments the level of this reservoir was held constant at 8 cmH_2O. The pressure of ejection was measured *via* a sidearm from the output cannula and stroke volume was collected by overspill from the output reservoir. The details of this procedure and the equipment used are given by Smith (1985*a*). After dissection, the hearts were perfused for 1–2 h prior to the experiment. Heart rates were measured over a 3-min period and stroke volumes represent the mean value for between 15 and 20 contractions. A second header tank feeding into the input reservoir *via* a three-way switch allowed rapid turnover to different perfusates without interrupting the perfusion of the heart. Myogram recordings were made using a suction electrode configuration (Smith, 1985*a*) and a Grass low-level d.c. preamplifier (7P1B). All hard copy, of both pressure and electrical recordings, was made using a Grass Polygraph 79C fitted with d.c. driver amplifiers (7DA). This gave a curvilinear output.

Solutions of 5-HT (Sigma Chemical) were made from a freshly prepared 10^{-3} mol l^{-1} stock solution made up in filtered sea water. Both the normal perfusate (filtered sea water) and test solutions flowed into the heart and into the experimental chamber. The rate of flow from the header tank into the input reservoir was approximately 1 l every 10 min. The experimental chamber had a volume of 50 ml and observations with dye solution indicated a rapid and even flush-out. The volume of the input cannula was 1·9 ml. At all concentrations of 5-HT the hearts were initially perfused at an input pressure of 7·5 cmH_2O which gave an average stroke volume of approximately 0·566 ml. Measurements of heart rate, stroke volume and pressure pulses were made at least 3–4 min from either a change in the input pressure or the switching over to a new concentration of 5-HT.

A total of 10 hearts was used in these experiments. Where the treatment was the same, the results were pooled after correction for the different heart weights. The correction for heart weight was a simple arithmetical one, expressing output per gram ventricular wet weight. A more extensive study on 30 hearts perfused at the same pressure (P1 = 7·5; P2 = 8 cmH_2O) suggests that the relationship between heart weight (Hwt) and stroke volume (SV) is not this simple [P. J. S. Smith, unpublished data: over a range of heart weights (0·15–0·65 g), the relationship approximates to a power curve with the equation $SV(ml) = 2{\cdot}85 \times Hwt^{1{\cdot}73}$; $r^2 = 0{\cdot}81$].

RESULTS

Data from the study by Smith (1985*a*) show that the *Busycon* heart responds to regulation of the preload with linear changes in stroke volume and with a change in heart rate that fits an apparent exponential function. When the heart is perfused with filtered sea water and the pooled data from the preparations are corrected for heart

weight, the stroke volume response in the present study is linearly related to the level of the preload, at a constant afterload of 8 cmH_2O (Fig. 1). Increase in the heart rate with increases in preload begins to level off at around 16 beats min^{-1} (Fig. 2). Subsequent perfusion with an increasing concentration series of 5-HT did not cause any clear change in the stroke volume response as shown in the pooled data (Fig. 1). Heart rate is affected by the application of 5-HT but the dose dependency, particularly at lower preload values, is not clear. This is partly because variation between the preparations introduces considerable deviation about the mean, but also because even the higher concentrations have only a limited effect on the rate. At filling pressures approximately equivalent to those expected *in vivo* (P1 = 2–4 cmH_2O), the heart rate increases by only 20% even when perfused with 5×10^{-8} mol l^{-1} 5-HT. It would appear from these data that 5-HT has a limited effect on cardiac output.

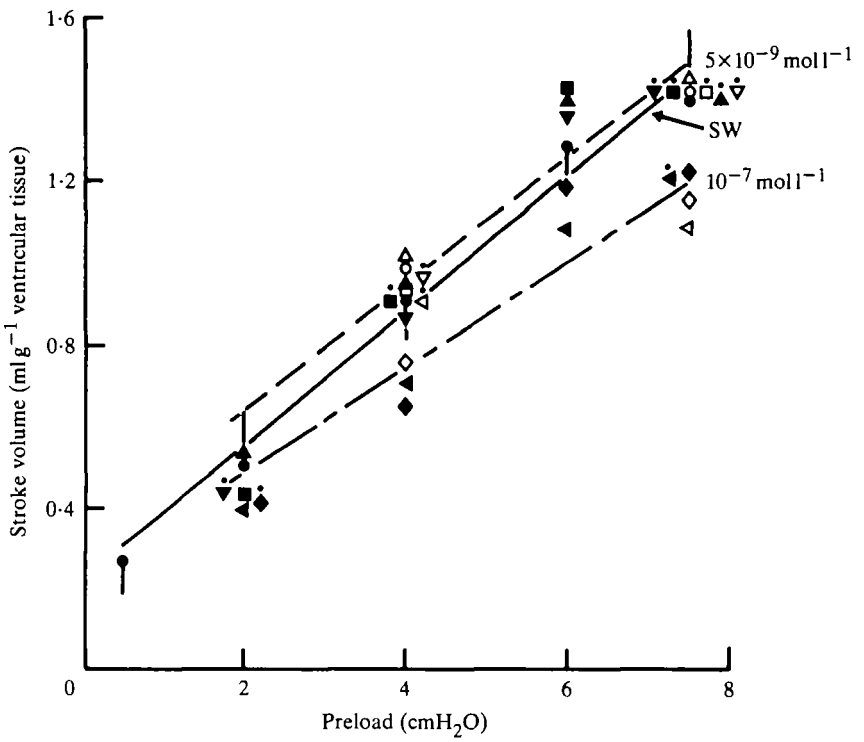

Fig. 1. Relationship between the input pressure, 5-hydroxytryptamine (5-HT) concentration and stroke volume for the ventricle of *Busycon canaliculatum*. Increasing concentrations of 5-HT appear to have a limited effect on the stroke volume. Regression lines are fitted through the data at concentrations of 5×10^{-9} and 10^{-7} mol l^{-1} as well as for perfusion with filtered sea water alone ($P < 0{\cdot}001$). The points are the mean results from five preparations with S.E. bars shown on the control run (SW). Filtered sea water, ●; 10^{-10}, ■; 5×10^{-9}, ▲; 10^{-8}, ▼; 5×10^{-8}, ◆; 10^{-7} mol l^{-1} 5-HT, ◀. Afterload was held at 8 cmH_2O. Perfusion started at high preload levels and the open symbols represent the returning performance levels. Symbols with dots above are overlapping values displaced to either side of the preload value.

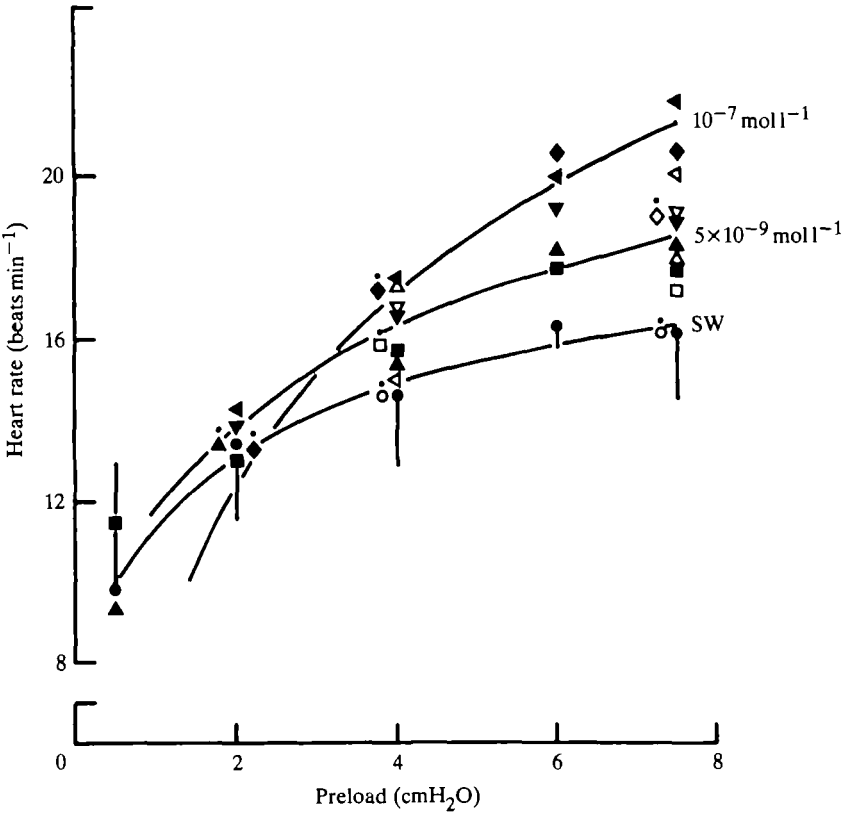

Fig. 2. Relationship between the input pressure, 5-hydroxytryptamine (5-HT) concentration and heart rate. Heart rate responds to 5-HT in a dose-dependent manner. Exponential curves (see Smith, 1985*a*) are fitted through the control run and those at 5×10^{-9} and 10^{-7} mol l^{-1} 5-HT. The points are the means of five preparations with S.E. bars on the control run. The error bars are large due to variation in heart rate between preparations. All preparations show the same trend in response to 5-HT. Symbols as in Fig. 1.

During these experiments it was observed that, although output changes were limited, the stroke volume was being ejected at a considerably higher flow rate. The result is graphically illustrated by examining the characteristics of the aortic pressure pulse in response to the different concentrations of 5-HT (Fig. 3). Only the pooled data from preparations perfused at a preload value of 6 cmH_2O are shown. As the concentration of 5-HT perfusing the heart was increased, the amplitude of the pressure pulse increased in a dose-dependent manner while the duration of the pressure pulse decreased. Approximately the same stroke volume was being ejected, but at a higher pressure and over a shorter period of time, thus increasing the rate of flow.

Using a series of concentrations of 5-HT over a range of pressures was a protracted experiment taking approximately 3–4 h to complete, after the initial 1–2 h settling period. Control experiments where hearts are perfused with sea water (Smith, 1985*a*) show that no deterioration occurs over this period. However, to test this, one

heart was perfused alternately with filtered sea water and different concentrations of 5-HT. The heart was perfused at only one preload level of 6 cmH_2O.

Pressure pulse amplitude and duration, as well as heart rate, behaved exactly as described in the previous series of experiments with the threshold of response around 5×10^{-9} $moll^{-1}$ 5-HT. When the heart is perfused with sea water, at 5-HT concentrations between 5×10^{-9} and 10^{-7} $moll^{-1}$, the values of these parameters return to control levels. Above 10^{-7} $moll^{-1}$, 5-HT has a persistent effect after the heart is returned to sea water. There is, however, some indication that the stroke volume is increased by the intermediate concentrations of 5-HT (Fig. 4). At a 5-HT concentration of 5×10^{-8} $moll^{-1}$, this increase was maximal – of the order of 50% over the original value. 5-HT also has a persistent effect, reducing the stroke volume after the heart is returned to sea water. The experimental regime used in the first series of experiments may not be ideal for demonstrating the regulation of stroke volume by 5-HT. However, the point remains that the most striking result of perfusing the hearts with this cardioexcitatory transmitter is its action on the pressure pulse parameters.

The response of the hearts to low concentrations of 5-HT was variable. In the majority of the preparations, the threshold for an effect on the pressure pulse

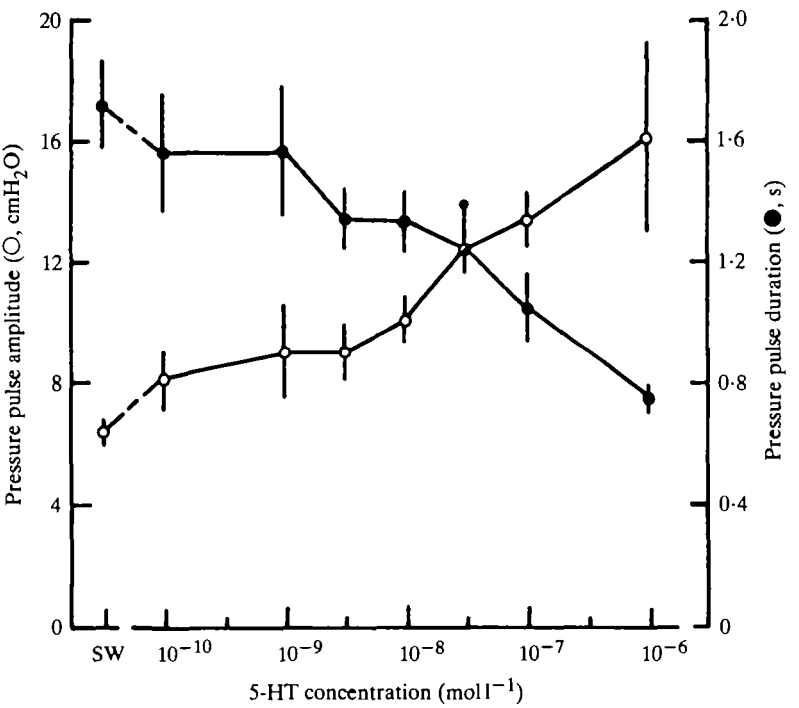

Fig. 3. Changes in the pressure pulse amplitude (○) and duration (●) in response to different concentrations of 5-hydroxytryptamine (5-HT) when the heart was perfused at a constant preload value of 6 cmH_2O; afterload was 8 cmH_2O. Standard deviations of the mean are shown for eight preparations. SW, sea water alone.

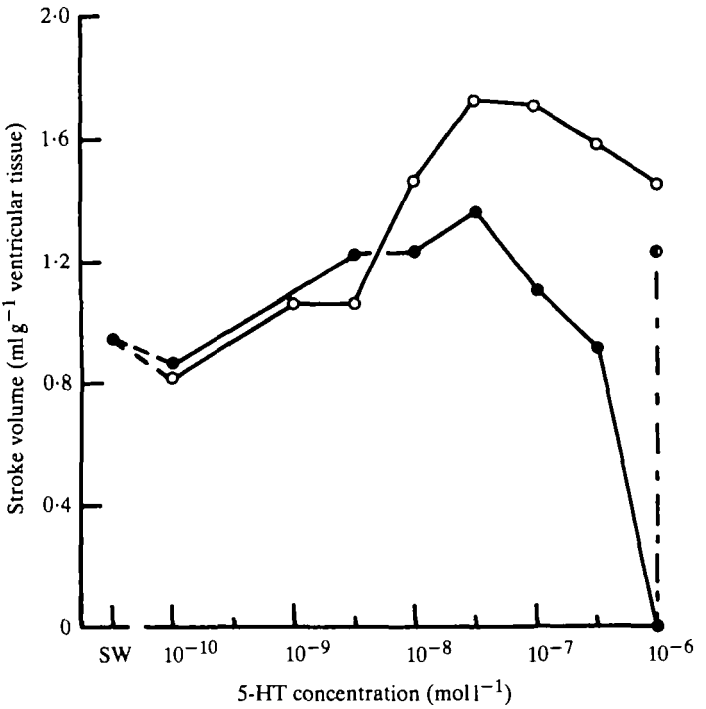

Fig. 4. Response of a single preparation to different concentrations of 5-hydroxytryptamine (5-HT) (○) at a single preload value of 6 cmH_2O; afterload was 8 cmH_2O. The heart was returned to sea water (SW) between 5-HT runs. Solid symbols are the output values in sea water after the corresponding run in 5-HT. The additional symbol at 10^{-6} mol l^{-1} is the value on return to 10^{-6} mol l^{-1} after perfusion with sea water.

amplitude and duration was between 5×10^{-9} and 10^{-8} mol l^{-1}. Heart rate may be increasing at concentrations of 10^{-9} mol l^{-1} and below (Fig. 2). One preparation showed a clear response in both pressure pulse characteristics and rate at a concentration of 10^{-10} mol l^{-1}. The example shown in Fig. 5, where the myogram and pressure pulse were recorded simultaneously, showed no effect at 10^{-10} mol l^{-1} but a clear response at 10^{-9} mol l^{-1} in both the parameters. A rate increase accompanies a more rapid rise in the prepotential and the spike component becomes clearer. The apparent reduction in the amplitude of the recording is most probably the result of a gradual loss of suction. It did not return to the control level when subsequently perfused with sea water. The most interesting response, however, with increasing 5-HT concentration is the reduction in the plateau duration at the same time that the amplitude of the pressure pulse is increasing. This was observed in all four of the preparations where the electrical activity was monitored. In the example shown in Fig. 6 the amplitude of the myogram increases considerably on application of 5-HT (5×10^{-7} mol l^{-1}) and returns to the control level on washing out the 5-HT. This preparation is already dependent on 5-HT for regular and strong contractions, as can be seen from the records before and after the application of 5-HT.

DISCUSSION

Data obtained in this study confirm previous evidence (Smith, 1985*a*) indicating autoregulation when preload is increased. A family of curves, obtained with a series of 5-HT concentrations, does not show a stroke volume change in the pooled data (Fig. 1) but does show a clear but limited change in heart rate (Fig. 2). Low concentrations of 5-HT alter the way in which the blood is ejected from the heart so

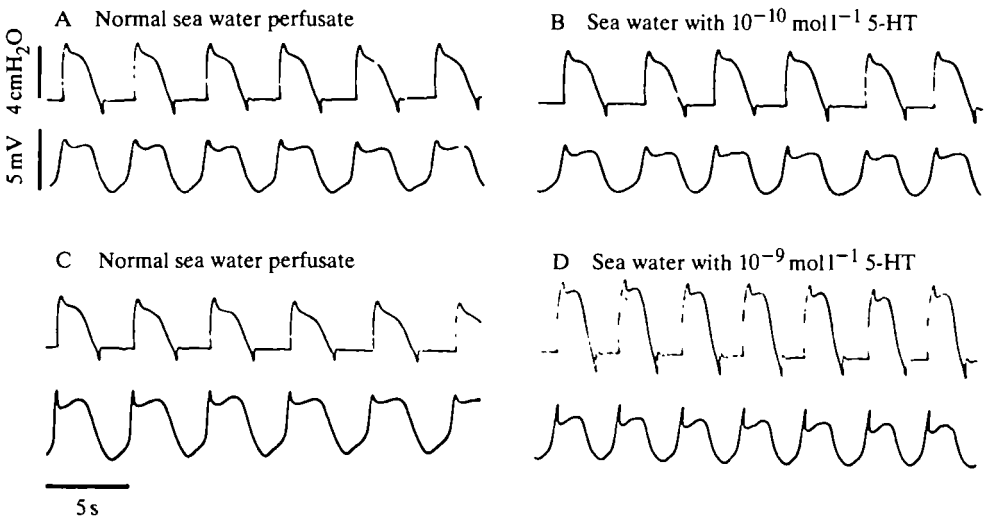

Fig. 5. Simultaneous recording of the aortic pressure pulse (upper record) and the ventricular myogram (lower record) at a preload level of 6 cmH_2O. (A) Control recording in filtered sea water; (B) perfused with 10^{-10} mol l^{-1} 5-hydroxytryptamine (5-HT); (C) control recording in filtered sea water; (D) perfused with 10^{-9} mol l^{-1} 5-HT.

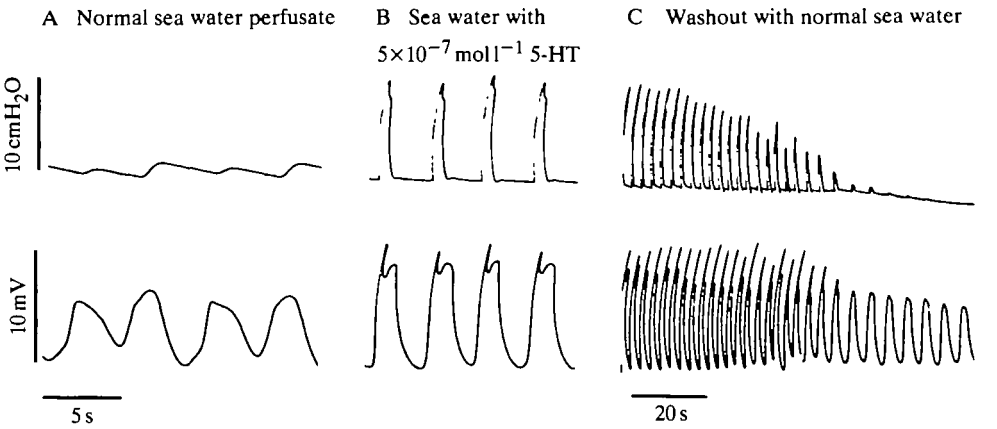

Fig. 6. Effect of perfusing the isolated beating heart with 5×10^{-7} mol l^{-1} 5-hydroxytryptamine (5-HT). (A) An example of reduced activity in sea water from a heart which contracted at the lower 5-HT concentrations shown in Fig. 5, but which had become 5-HT-dependent. (B) Performance after the application of 5×10^{-7} mol l^{-1} 5-HT. (C) 20 s after the return to sea water the amplitude of both the pressure pulse and the myogram begin to decline. The spike component of the latter eventually disappears.

as to increase the flow rate and pressure (Fig. 3). There is some evidence from a single preparation and different treatment that 5-HT may regulate stroke volume at intermediate concentrations (Fig. 4). Clearly, more experiments using the alternative perfusion technique are needed to decide the relevance of this particular result.

Suction electrode recording from the perfused beating ventricle of *Busycon canaliculatum* (Fig. 5) reveals a spike-and-plateau waveform very much like that seen in the perfused ventricle of another prosobranch, *Rapana thomasiana* (Hill & Irisawa, 1967). The effects of 5-HT on the myogram at concentrations below 10^{-9} mol l^{-1} are variable and concentrations of 10^{-7} mol l^{-1} and above have a persistent effect not reversible by washing. However, from 10^{-9} to 10^{-6} mol l^{-1} the relationship between spike and plateau induction and increased force is clear (Figs 5, 6). Increasing concentrations of 5-HT cause a dramatic increase in the amplitude of both the spike and plateau, with the duration of the latter being greatly reduced. This was somewhat unexpected since the duration of the plateau phase of the action potential is supposedly closely related to the force of the accompanying contraction in either the heart or cardiac muscle fibres. Matsui (1945, 1961) observed that stretch applied by increasing internal pressure enhanced the amplitude of beat in the isolated ventricle of *Dolabella auricularia*. Nomura (1963) made microelectrode recordings from single muscle fibres of the ventricle of *Dolabella auricularia*, while recording the force and applying stretch. Plateau duration was greater in a normally beating preparation than in a mechanically inactive one. Application of a stretch to a preparation between beats led to an increase in the duration of the plateau of the next action potential and a marked increase in force, while a sudden release of stretch between beats induced a shortened duration of the plateau in the next beat and a several-fold drop in force. Nomura (1963) concluded that the duration of the plateau of the cardiac action potential is closely related to the force of the accompanying beat. Similarly, Hill & Irisawa (1967) found that the action potential recorded from the isolated heart of *Rapana thomasiana* possessed a plateau which was sensitive to internal perfusion pressure. A sudden increase in pressure induced the immediate appearance of a plateau on the action potential and a marked increase in force. A sudden drop in pressure led to loss of the plateau and a marked drop in force.

At the moment we do not know why 5-HT in *Busycon canaliculatum* should increase the force of contraction, as measured by the amplitude of the pressure pulse, yet cause an apparently contradictory reduction in the duration of the plateau. It is worth noting, however, that the action of stretch on the myocardium, particularly as a result of internal perfusion (Smith, 1985*a*), is visibly different from the action of the neurotransmitter. Increasing internal pressure will, up to a limit, cause an increase in the end diastolic volume. In a control preparation perfused with sea water alone, it is clear that there is also an appreciable cardiac reserve at the end of systole. With the higher concentrations of 5-HT (approximately 10^{-7}–10^{-6} mol l^{-1}) it appeared that the heart ejected its entire volume and did not fill to nearly the volume previously observed. The effect of stretch and drug treatment may not therefore be comparable with regard to the mechanism of force generation. A more difficult contradiction lies in the difference between the results reported here

and those for *Dolabella auricularia* (Hill, 1974*a*). However, the response of the molluscan myocardium to cardioactive substances is notoriously unpredictable. Good examples of this can be found in the variable responses of bivalve species to the cardioactive tetrapeptide (Phe-Met-Arg-Phe) FMRFamide (Painter, Price & Greenberg, 1982) and the neurotransmitter acetylcholine (Greenberg, 1965, 1970). 5-HT can even be inhibitory in some cases (for example, *Modiolus demissus*; Wilkens & Greenberg, 1973). Perhaps what we are seeing in this study is variation expressed at the level of the action potential.

In the preparation described here there is no clear effect of 5-HT on stroke volume, but there is a dose-dependent effect on heart rate. 5-HT increases pressure of ejection, but as the contents of the heart are ejected more rapidly there need not be a change in stroke volume. Thus there is a very clear pattern, in which increasing concentrations of 5-HT increase the output pressure pulse in each beat but decrease the pulse duration. A sharp increase in flow rate is then accompanied by a relatively constant stroke volume. In an intact animal this could provide for moving approximately the same volume of blood out against an increased peripheral resistance. Soft-bodied animals such as molluscs, where the blood system is an integral part of the hydrostatic skeleton, face a particular problem with increasing vascular resistance during muscular contraction. In some circumstances, such as exercise in cephalopods, cardiac output and peripheral resistance will rise together (see Smith, 1985*b*) but there will be conditions in the gastropod where resistance rises when there is little need for increased cardiac output; for example, when contracting into the shell or even during normal locomotory activity. Under such conditions peripheral resistance will go up and a higher pressure of ejection will be needed to circulate the blood. High ejection pressure might be particularly important during emergence from the shell.

In vivo, a pattern of neuronal activity causing the release of 5-HT may increase beat-by-beat output, pressure and velocity of blood flow. If the effects of perfusion with 5-HT in solution are compared to the serotonergic components of neural regulation, it may be possible to predict the role of neural regulation in the cardiac response to altered haemodynamic conditions.

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